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Cloning, characterization and tissue expression of rat SULT2B1a and SULT2B1b steroid/sterol sulfotransferase isoforms: Divergence of the rat *SULT2B1* gene structure from orthologous human and mouse genes

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Abstract

cDNAs for rat SULT2B1 steroid/sterol sulfotransferase isoforms were cloned, and the encoded proteins overexpressed, purified and characterized. The rat SULT2B1a isoform avidly sulfonates pregnenolone but poorly utilizes cholesterol as a substrate, whereas cholesterol is more efficiently sulfonated than pregnenolone by the SULT2B1b isoform; on the other hand, neither isoform sulfonates dehydroepiandrosterone to any significant degree. Real-time PCR revealed that SULT2B1a was only expressed in brain and testis, whereas SULT2B1b was mainly expressed in skin, intestine and kidney. The *SULT2B1* gene is unique among steroid/sterol sulfotransferase genes in that it encodes for two isoforms as a result of an alternative exon I. Interestingly, whereas the orthologous human and mouse *SULT2B1* gene structures are identical, the rat *SULT2B1* gene structure diverges. Similar to human and mouse *SULT2B1* genes the rat *SULT2B1* gene consists of an alternative exon I; however, as a result of exonic rearrangement, the genic locations of exons IA and IB are reversed in the rat gene. Where exon IA is located downstream of exon IB in the human and mouse *SULT2B1* genes, in the rat *SULT2B1* gene exon IA is located upstream of exon IB. Furthermore, unlike the case with human and mouse *SULT2B1* genes where differential splicing is necessitated since a portion of exon IA is fused with exon IB to complete the SULT2B1b mRNA, this step is not required with the rat gene.
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1. Introduction

The superfamily of cytosolic sulfotransferases (SULT) includes two steroid/sterol sulfotransferase subfamilies, i.e. SULT2A1 and SULT2B1 (Nagata and Yamazoe, 2000). In contrast to the SULT2A1 subfamily [commonly referred to as dehydroepiandrosterone (DHEA) sulfotransferase], which exists as a single protein with a broad substrate predilection, the SULT2B1 subfamily consists of two isoforms (SULT2B1a and SULT2B1b) that exhibit a more selective substrate preference. In human and mouse species, the SULT2B1 isoforms are derived from a single gene as a result of an alternative exon I and differential splicing (Her et al., 1998; Shimizu et al., 2003). The SULT2B1b isoform in both human and mouse species efficiently sulfonates cholesterol, whereas the SULT2B1a isoform avidly sulfonates pregnenolone, while neither isoform sulfonates DHEA efficiently (Javitt et al., 2001; Fuda et al., 2002; Shimizu et al., 2003). There is a significant differential expression of the *SULT2A1* and *SULT2B1* genes in the mouse that is particularly striking during embryonic development (Shimizu et al., 2003). Additionally, there is selective tissue expression of the SULT2B1 isoforms. For

Abbreviations: SULT, sulfotransferase; PCR, polymerase chain reaction; UTR, untranslated region; bp, base pair; kb, kilobase; PAPS, 3′phosphoadenosine 5′-phosphosulfate; RACE, rapid amplification of cDNA ends; GST, glutathione-*S*-transferase; TLC, thin layer chromatography; DHEA, dehydroepiandrosterone.

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instance, in the mouse, SULT2B1a is selectively expressed in the central nervous system. The significance of this finding is that SULT2B1a actively sulfonates pregnenolone and pregnenolone sulfate is an important neurosteroid in the rodent brain (Alomary et al., 2001; Baulieu et al., 2001; Engel and Grant, 2001; Plassart-Schiess and Baulieu, 2001). On the other hand, the SULT2B1b isoform, in both mice (Shimizu et al., 2003) and humans (Higashi et al., 2004), is selectively expressed in skin where cholesterol sulfate plays an important role in development and maintenance of the epidermal barrier (Strott and Higashi, 2003). Based on the finding of tissue-specific expression of the SULT2B1 isoforms in the mouse, we wanted to further explore selective expression as well as functionality of the SULT2B1 isoforms in the rat with a particular interest in expression of these steroid/sterol sulfotransferases in brain and skin tissue. Thus, we cloned and characterized the rat *SULT2B1* gene and gene products, and in so doing discovered that rat *SULT2B1* gene structure diverged from the orthologous human and mouse genes, which are identical.

2. Materials and methods

2.1. Materials

Cholesterol, sulfated steroids, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), 2-hydroxypropyl-β-cyclodextrin and iodine crystals were obtained from Sigma. ^{3}H-cholesterol (60 Ci/mmol) was from American Radiolabeled Chemicals. ^{3}H-pregnenolone (14 Ci/mmol) and ^{3}H-DHEA (60 Ci/mmol) were purchased from PerkinElmer Life Sciences. Silica gel thin-layer chromatography (TLC) plates were procured from Analtech and organic solvents were obtained from Mallinckrodt-Baker.

2.2. Cloning of rat SULT2B1a and SULT2B1b cDNAs

Degenerate primers were designed based on conserved regions of human SULT2B1a (GenBank accession no. U92314), human SULT2B1b (GenBank accession no. U92315), mouse SULT2B1a (GenBank accession no. AF478566) and mouse SULT2B1b (GenBank accession no. AF026072).

To establish the mid-region, PCR with Platinum *Taq* DNA Polymerase (Invitrogen) was performed using sense (5′–GGNGARTAYTTYMGNTAYAARGG–3′) and antisense (5′–CATNGTRTTNGCYTTCATNGC–3′) primers with the Marathon-Ready™ rat (Sprague-Dawley) brain cDNA (BD Biosciences Clontech) as template. Nested PCR was carried out using the first PCR product as template and the sense (5′–GCNCCNTGGTGYGARAC–3′) and antisense (5′–CCANCCYTTDATRTGRTCRAACC–3′) primers. PCR conditions were: denaturing at 94 °C for 5 min, followed by 30 cycles of denaturing at 94 °C for 30 sec, annealing at 37 °C for 30 sec and extension at 72 °C for 30 sec. PCR products were purified, subcloned into pCR2.1-TOPO vector using the TOPO TA Cloning kit (Invitrogen) and sequenced.

To establish the 3′-untranslated region (UTR), the 3′-rapid amplification of cDNA ends (RACE) method was employed (BD Biosciences Clontech). Briefly, PCR was performed using Marathon-Ready™ rat brain cDNA as template, a gene-specific sense primer (5′–GGCCAAGGTGATTTACTTG–3′) and adaptor primer AP1 (5′–CCATCCTAATACGACTCACTATAGGGC–3′). Nested PCR was carried out using the first PCR product as template and another pair of gene-specific sense (5′–AGATTGCTGTACAATTAAAG–3′) and nested adapter AP2 (5′–ACTCACTATAGGGCTCGAGCGGC–3′) primers. PCR conditions were: denaturing at 94 °C for 5 min, followed by 30 cycles of denaturing at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 2 min. PCR products were analyzed by electrophoresis using 2% agarose gels. Gel-purified products were subcloned into pCR2.1-TOPO vector and sequenced.

To establish the 5′-UTR, 5′-RACE was employed using the SMART™ RACE cDNA Amplification Kit (BD Biosciences Clontech). Briefly, a mixture of either 3 μg rat brain or rat skin total RNA (OriGene) and SMART II™ A oligonucleotide was used to carry out a reverse transcription reaction using either a 5′-RACE CDS primer that is provided with the kit, or gene-specific primer A (5′–CGGATCCACGAGGGGTCCCCATC–3′). PCR was performed using both templates and combinations of universal primer mixture (UPM) and gene-specific primers A or B (5′–CGGCCAGACTGAGGCTCTCTGGTG–3′). Nested PCR was carried out using the first PCR product and combination of UPM and gene-specific primers B or C (5′–GCCGACTGGAAAGGGAATGCC–3′). PCR conditions were: denaturing at 94 °C for 5 min, followed by 30 cycles of denaturing at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 2 min. After gel purification, PCR products were subcloned into pCR2.1-TOPO vector and sequenced.

2.3. Construction of rat SULT2B1a and SULT2B1b expression vectors

A mixture of either rat (Sprague-Dawley) brain or skin total RNA (OriGene) and SMART II™ A oligonucleotide was subjected to reverse transcription using the 5′-RACE CDS primer. PCR was carried out using gene-specific sense (5′–GTCGACATGTCTCCATGGTCCAGGAATACC–3′) and antisense (5′–GCGGCCGCTTATTGTGAGGATCCTGGGTTGG–3′) primers with skin-derived cDNA as template. For brain-derived templates, PCR was carried out using gene-specific sense (5′–ACACAGAGCTGCAGACACAC–3′) and antisense (5′–GGGGGGAGTGACAAG–3′) primers, after which nested PCR was carried out using sense (5′–GTCGACATGGACGGGCCACAGCCCCCGGCCCTATG–3′) and antisense (5′–GCGGCCGCTTATTGTGAGGATCCTGGGTTGG–3′) primers. Underlines indicate *Sal*I and *Not*I restriction sites, respectively. PCR conditions were: denaturing at 94 °C for 5 min, followed by 30 cycles of denaturing at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 2 min. After gel purification, PCR products were subcloned into pCR2.1-TOPO vector and sequenced. cDNAs with correct restriction sites and translation into the precise amino acid sequence were digested with *Sal*I and *Not*I and ligated into *Sal*I/*Not*I-

digested pGEX-6P-3 vector using Quick Ligation™ Kit (New England Biolab).

2.4. Generation of recombinant rat SULT2B1a and SULT2B1b

Glutathione-*S*-transferase (GST) gene fusion system (GE Healthcare) was employed for purification of bacterially overexpressed recombinant proteins. Briefly, plasmids were used for transformation of BL21-Gold (DE3) pLysS (Stratagene). Vectors were transformed to competent cells, then the bacterial cultures were added to 100 ml Luria–Bertani broth and grown overnight at 26 °C in ampicillin medium to minimize expressed proteins in inclusion bodies. Additional Luria–Bertani broth containing ampicillin was then added to increase culture volumes to 1 liter, and incubations were continued until the OD_{595nm} reached 0.6 at which time isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 50 μM. After overnight incubation, cells were collected by centrifugation, and the bacterial pellets were frozen at −80 °C. Pellets were extracted by sonication in iced PBS to which had been added a protease inhibitor cocktail tablet (Roche Molecular Biochemicals) and 1 mg/ml lysozyme (Sigma). The sonication step was carried out for 1 min and repeated 5 times on ice. Extracts were centrifuged at 204,000×*g* for 1 h at 4 °C. Supernatants were collected, mixed with 1 ml glutathione Sepharose 4B resin (GE Healthcare) and incubated for 1 h at 4 °C. The resin mixture of GST-fusion protein was transferred to a plastic column and washed with a 25-column volume of cleavage buffer [50 mM Tris–HCl (pH 7.0), 150 mM NaCl, 1 mM dithiothreitol]. 40 μl (80 U) of PreScission Protease (GE Healthcare) in 1 ml cleavage buffer were applied per 1 ml column resin and incubated overnight at 4 °C. After elution, protein concentrations were determined with the BCA Protein Assay kit (Pierce Chemical) using BSA as standard. To check the quality of recombinant proteins, SDS–PAGE was performed using a NuPage 12% Bis–Tris gel (Invitrogen) and a standard procedure.

2.5. Kinetic analyses of rat SULT2B1a and SULT2B1b

Sulfotransferase activity was determined using tritium-radiolabeled steroid/sterol substrates. A 20-μl reaction vol. contained a specific substrate, 0.1 mM PAPS and a purified enzyme preparation as described above: SULT2B1a (1.2 μg) and SULT2B1b (0.1 μg) in the cholesterol assay; SULT2B1a (2.0 μg) and SULT2B1b (0.4 μg) in the pregnenolone assay; SULT2B1a (2.8 μg) and SULT2B1b (0.4 μg) in the DHEA assay, in 0.1 mM Tris–HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, 0.2 mM 2-hydroxypropyl-β-cyclodextrin, and 4% ethanol (v/v). Reactions were carried out at 37 °C for 5 min and stopped at 100 °C for 5 min. After adding 10 μl of 5 mg/ml of a respective sulfoconjugated standard as carrier, 5 μl aliquots were applied to silica gel-TLC plates, and chromatography was carried out using the solvent system of chloroform/methanol/acetone/acetic acid/water (8:2:4:2:1). After development, TLC plates were dried and exposed to iodine vapor to visualize the location of the sulfoconjugated products. The iodine-adsorbed spots were excised, the silica was placed into counting vials containing 5 ml scintillation cocktail, and the radioactivity was determined by liquid scintillation spectrometry.

2.6. mRNA expression analysis of rat SULT2B1a and SULT2B1b by real-time PCR

Total RNA isolated from rat prostate and placenta was obtained from BD Biosciences Clontech, whereas total RNA prepared from other rat tissues was obtained from OriGene Technologies. Reverse transcription (RT) was performed using SuperScript™ III (Invitrogen) according to the manufacturer's instructions. Briefly, using 5 μg total RNA as template, first-strand cDNA was made using 25 pmol oligo(deoxythimidine)$_{20}$ and 25 ng random hexamer primer in a 20-μl reaction vol. After heat denaturing at 65 °C for 5 min, RT was carried out at 25 °C for 10 min and then 50 °C for 50 min.

Real-time PCR was performed using a fluorescence temperature cycler (AB 7500 Real-Time PCR System) and SYBR Green I as a double-stranded DNA-specific binding dye, according to the manufacturer's instructions (Applied Biosystems). This system uses fluorescent-based PCR chemistries to provide quantitative detection of nucleic acid sequences using real-time analysis. Amplifications were carried out using 12.5 μl of 2× SYBR Green PCR Master Mix, which contains SYBR Green I dye, Ampli*Taq* Gold DNA Polymerase, deoxynucleoside triphosphates with deoxyuricil triphosphate, Passive Reference and optimized buffer components (Applied Biosystems), 0.5 μl of each primer, 2 μl of cDNA in a total vol. of 25 μl. Real-time PCR conditions were: activation of DNA polymerase at 95 °C for 10 min, followed by 40 cycles of melting at 95 °C for 15 sec and annealing and extension at 60 °C for 1 min. SYBR Green I fluorescence was detected at the end of each cycle to monitor the amount of PCR product formed during the cycle. A dissociation curve analysis of the amplification products was performed at the end of the PCR run by rapidly increasing the temperature to 95 °C followed by immediate cooling to 60 °C for 1 min, after which the temperature was gradually increased to 95 °C with continuous measurement of fluorescence to confirm amplification of specific transcripts. The melting temperature profile for all samples of SULT2B1a and SULT2B1b, as well as β-actin demonstrated a single peak at 83, 83, and 88 °C, respectively. The overall results obtained were analyzed by the 7500 System Sequence Detection Software Version 1.2.3 (Applied Biosystems).

Primer sequences used were: 5′–TCACTGGAGAAACTGAGGCAGG–3′ (sense) and 5′–GGAAGGCTGAAGGCACTTATGG–3′ (antisense) for SULT2B1a, 5′–ATGGGGCTCATTGGAGAACAG–3′ (sense) and 5′–TGGAAGGCTGAAGGCACTTATG–3′ (antisense) for SULT2B1b. Primers were designed to recognize a different exon in each gene; furthermore, the appropriate PCR products were verified by agarose gel electrophoresis. Full-length sequences of rat SULT2B1a and rat SULT2B1b, which were subcloned to pCR2.1-TOPO vector as described above were used as external cDNA standards for SULT2B1a and

SULT2B1b. The concentration of each standard was determined by measuring the $OD_{260\ nm}$ and the copy number calculated.

Rat β-actin was quantified to normalize SULT2B1a and SULT2B1b mRNA levels, and the final results are expressed as the ratio of copy number of a specific SULT2B1 mRNA to the copy number of β-actin. Primer sequences for β-actin were: 5′–GCGAGTACAACCTTCTTGCAGCTC–3′ (sense) and 5′–GCCAAATCTTCTCCATATCGTCC–3′ (antisense). External cDNA standards for β-actin were produced by inserting PCR products, which were generated using the primers as noted above and rat liver cDNA as a template into the pCR2.1-TOPO vector employing the TOPO TA Cloning kit. Vector constructs were used to transform to TOP10 (Invitrogen), and plasmid DNA was prepared by QIAprep Spin Miniprep Kit (QIAGEN). The appropriate size of PCR products was verified by agarose gel electrophoresis.

2.7. Amino acid sequence analysis

Multiple alignment analysis was carried out using the MacVector 7.0 system (Oxford Molecular, Madison, WI), which is based on the Clustal W algorithm (Thompson et al., 1994).

3. Results

3.1. Cloning of rat SULT2B1a and SULT2B1b cDNAs

Rat SULT2B1a cDNA (GenBank accession no. AY827147) and rat SULT2B1b cDNA (GenBank accession no. AY827148) consist of 1256 and 1125 nucleotides, respectively. The 5′-UTR lengths as determined by the 5′-RACE procedure are 77 and 51 nucleotides for SULT2B1a and SULT2B1b, respectively. SULT2B1a cDNA encodes for a protein of 375 amino acids with a calculated molecular weight of 42 178 and a p*I* of 5.93. SULT2B1b cDNA encodes for a protein of 340 amino acids with a calculated molecular weight of 38319 and a p*I* of 5.12. Since SULT2B1a and SULT2B1b are derived from a single gene as a result of an alternative exon I, the two isoforms are identical except for their amino termini; the unique amino terminus of SULT2B1a consists of 55 amino acids, whereas the unique amino terminus of SULT2B1b consists of 20 amino acids.

3.2. Rat SULT2B1 gene structure

Rat BLAT Search (http://genome.ucsc.edu/cgi-bin/hgBlat) is a web-based source of UCSC (University of California at Santa Cruz) Genome Bioinformatics for mapping target sequences to the genome. Using this program submitted rat SULT2B1 cDNA sequences matched precisely to bases in the rat genomic sequence and as a result the rat *SULT2B1* gene structure could be determined (Fig. 1A). Rat SULT2B1a is encoded by exon IA and exons II–VII, whereas SULT2B1b is encoded by exon IB and exons II–VII. All rat *SULT2B1* exon–intron splice junction patterns conform to the gt–ag rule (Mount, 1982), as indicated in Fig. 1B.

In contrast to the structures of orthologous human and mouse *SULT2B1* genes, which are essentially identical, the arrangement of exons IA and IB is altered in the rat *SULT2B1* gene. That is, as a result of exonic rearrangement, the locations of

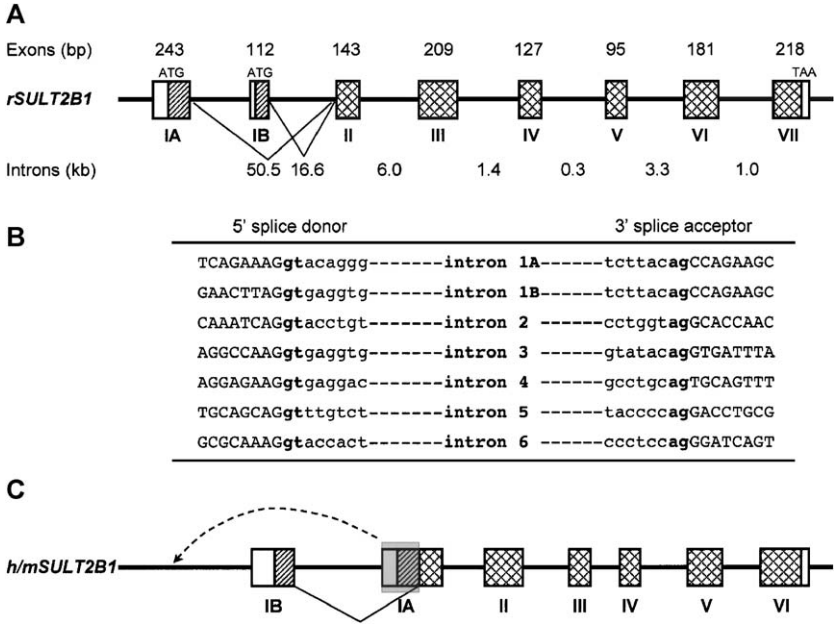

Fig. 1. (A) Schema of the rat (r) *SULT2B1* gene structure. Exons are labeled in bold Roman numerals. Exon lengths are given in bp, whereas intron lengths are in kb, as indicated above and below the schema, respectively. Open boxes in exons IA, IB and VII represent the 5′- and 3′-untranslated sequences; hatched areas in exons IA and IB represent unique amino acid coding sequences, whereas cross-hatched boxes in exons II–VII represent common amino acid coding sequences. (B) Exon/intron splice-junctions. All splice-junctions adhere to the **gt/ag** rule as indicated with bold type. (C) Schema of the human (h) and mouse (m) *SULT2B1* gene structure. General description is the same as for the rat *SULT2B1* gene. The shaded area indicates the portion of exon IA that is translocated in the rat gene; the broken arrow indicates directionality.

exons IA and IB are reversed in the rat *SULT2B1* gene. In the human and mouse *SULT2B1* genes exon IA is located downstream of exon IB as shown in Fig. 1C, whereas in the rat *SULT2B1* gene exon IA is located upstream of exon IB as depicted in Fig. 1A. Furthermore, only that portion of exon IA, which encodes for the unique amino terminal region of the SULT2B1a isoform is cleaved and translocated (cf. Fig. 1C). The portion of exon IA that is not translocated and which encodes for an amino acid sequence common to both SULT2B1 isoforms now becomes exon II in the rat gene with the total number of exons increasing from 7 to 8 that are found in the human and mouse *SULT2B1* genes.

3.3. Amino acid analysis of rat SULT2B1 isoforms and comparison to mouse and human counterparts

Figs. 2 and 3 display the amino acid alignment of rat, mouse and human SULT2B1a and SULT2B1b isoforms, respectively. The SULT2B1a and SULT2B1b proteins are, correspondingly, 65% and 70% identical. In Figs. 2 and 3, the unique amino termini of the respective SULT2B1a and SULT2B1b isoforms are set off by an I-bar. In contrast to the unique human SULT2B1a amino terminus, which consists of 8 amino acids, the unique amino termini of both rodent SULT2B1a isoforms are substantially longer at 55 and 54 amino acids for the rat and mouse versions, respectively (Fig. 2). On the other hand, the lengths of the unique amino termini of the SULT2B1b isoforms of the three species are similar (Fig. 3) and, interestingly, in contrast to the case with the SULT2B1a isoforms, the unique amino termini of the rat, mouse and human SULT2B1b isoforms are also highly similar (Fig. 3). Furthermore, those residues found by mutational analysis to be essential for cholesterol catalysis by human SULT2B1b (Fuda et al., 2002) are conserved in the rat and mouse (cf. Fig. 3).

In Figs. 2 and 3, as was done with the unique amino termini, the extended carboxy-terminal regions of all SULT2B1 proteins are also set off by an I-bar. As can be seen, the reverse of the situation with the SULT2B1a amino termini is illustrated, i.e. the carboxy terminus of the human SULT2B1 isoforms is considerably longer than that of the carboxy termini of the rodent species (cf. Figs. 2 and 3). Nevertheless, a characteristic of the extended carboxy termini of the three species is that they are proline-enriched; the significance of this feature is not yet understood. Finally, it is important to note that key amino acid residues involved in the binding of the universal sulfonate donor molecule, PAPS, as determined by the crystal structure of human and mouse estrogen sulfotransferase (Kakuta et al., 1997; Pedersen et al., 2002) and human hydroxysteroid sulfotransferase SULT2A1 (Pedersen et al., 2000) are completely conserved in the SULT2B1a (Fig. 2) and SULT2B1b (Fig. 3) proteins of the three species.

3.4. Kinetic analyses of rat SULT2B1a and SULT2B1b

A steady-state analysis of the rat SULT2B1 isozymes using cholesterol, pregnenolone and DHEA as substrates was carried out. K_m and V_{max} values were determined by Lineweaver–Burk transformations. The SULT2B1a isoform avidly sulfonates

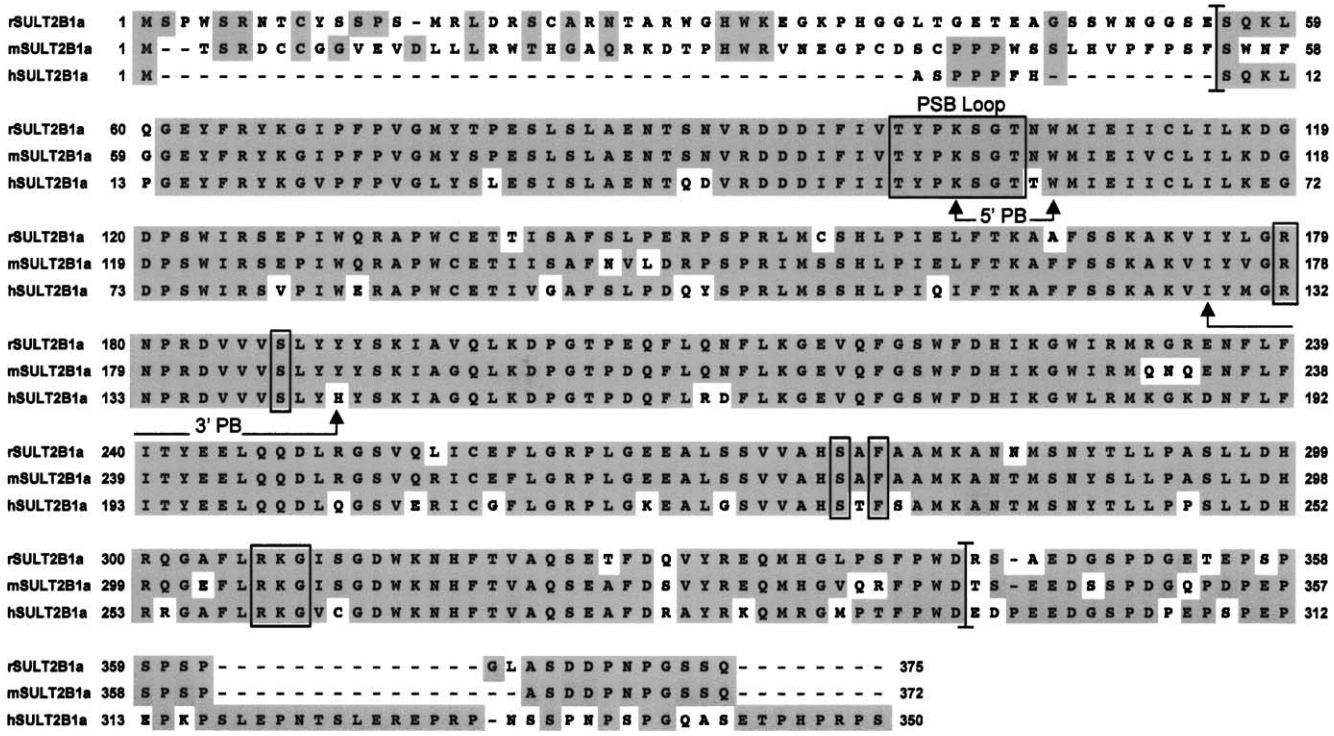

Fig. 2. Amino acid sequence alignment of rat (r), mouse (m) and human (h) SULT2B1a isoforms. Shaded amino acid residues denote identities and similarities, whereas residues within the boxes specify amino acids that are conserved in all cytosolic sulfotransferases involved in the binding of the PAPS co-substrate. The nucleotide-binding PSB loop is specified, and the 5′- and 3′-phosphate-binding motifs (5′PB and 3′PB) are delineated by arrows beneath the amino acid sequence. The I-bars isolate the extended amino- and carboxy-terminal ends.

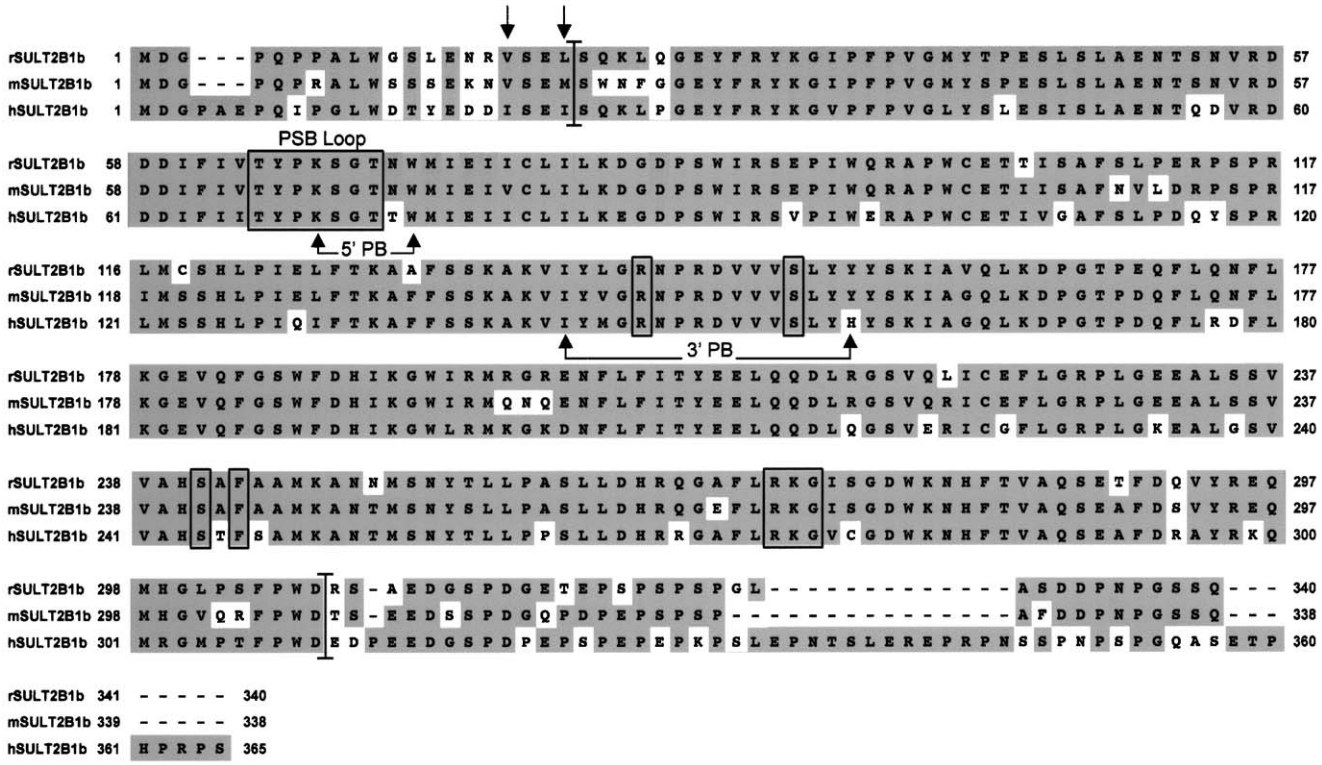

Fig. 3. Amino acid sequence alignment of rat (r), mouse (m) and human (h) SULT2B1b isoforms. General description is the same as in Fig. 2. Arrows above amino acid sequences at the amino termini indicate conserved residues essential for cholesterol catalysis.

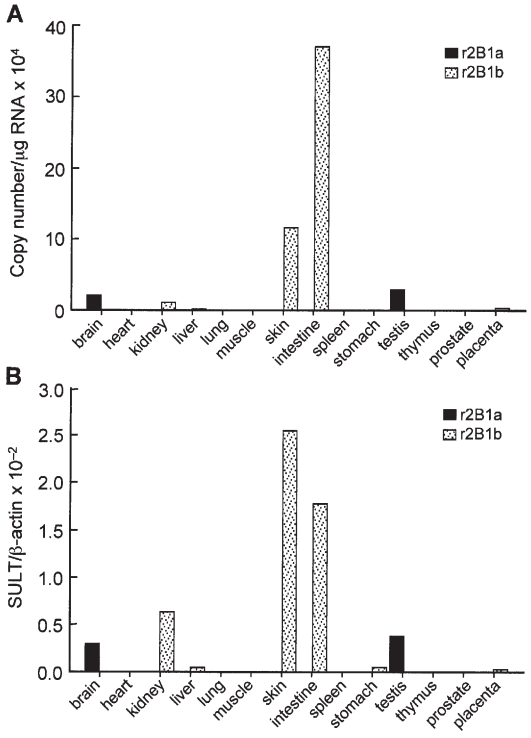

Fig. 4. Expression of rat (r) SULT2B1a mRNA (solid columns) and SULT2B1b (hatched columns) in various tissues, as determined by real-time RT–PCR: (A) mRNA copy number of each SULT per μg total RNA; (B) ratio of mRNA copy number of each SULT to mRNA copy number of β-actin.

pregnenolone, whereas DHEA is poorly sulfonated by this isoform, and cholesterol is essentially not sulfonated at all. The K_m values for pregnenolone and DHEA as substrates for the SULT2B1a isoform are respectively, 8.9 μM and 17.9 μM, whereas the K_m value for cholesterol was uncertain because of weak activity. Although the rat SULT2B1b isoform sulfonates pregnenolone more vigorously than cholesterol, it does so with an efficiency that is about half that of cholesterol, i.e. the V_{max}/K_m ratio for pregnenolone is 1.8, whereas for cholesterol it is 3.7; as with SULT2B1a, DHEA is a poor substrate for the SULT2B1b isoform. The cholesterol K_m value (1.7 μM) as substrate for the SULT2B1b isoform is 6- to 12-fold lower than that for pregnenolone and DHEA.

3.5. mRNA expression of rat SULT2B1a and SULT2B1b

Multiple rat tissues were examined by real-time PCR for expression of the SULT2B1 isoforms as illustrated in Fig. 4. Expression of the SULT2B1a isoform was found in only the brain and testis where the degree of expression was more or less equivalent. SULT2B1b expression was prominent in the skin and intestine, modest in the kidney and weak in liver, stomach and placenta (Fig. 4).

4. Discussion

The *SULT2B1* gene is unique among the steroid/sterol sulfotransferase genes in that it encodes for two isoforms as

a result of an alternative exon I. The two isoforms are thus identical except for unique amino-terminal ends. Additionally, in the human and mouse species, differential splicing is also involved since a portion of exon IA encoding for an amino acid sequence common to both isoforms is fused with exon IB to complete the SULT2B1b mRNA (cf. Fig. 1C). The structure of the rat *SULT2B1* gene demonstrates an interesting variation on this theme, a finding that was unexpected and which created some concern at first since it was thought not unreasonable to expect that the structure of the rat *SULT2B1* gene would be similar to the human and mouse *SULT2B1* genes whose structures are alike, particularly since rat and mouse species are more closely related than human and mouse species. Certainly, regarding the SULT2B1 proteins, rat and mouse SULT2B1a (78%) and SULT2B1b (87%) are significantly more similar than the rat and human SULT2B1a (68%) and SULT2B1b (72%) proteins. However, what is especially interesting concerning the rearrangement of the rat *SULT2B1* gene is that there is not just a relocation of exon IA to be upstream of exon IB, which is the reverse of the situation in human and mouse genes, but that only that portion of exon IA encoding for the unique amino terminus of the SULT2B1a isoform is relocated. This is opportune for otherwise the SULT2B1b protein would sustain a substantial amino acid deletion, which presumably would render it inactive [e.g. it would lack the critical PSB loop (cf. Fig. 3)]. The part of exon IA encoding for common amino acid sequence of the two isoforms remains in the same relative gene position as it is in the human and mouse genes and becomes exon II (cf. Fig. 1A/C). As a result, differential splicing, which is necessitated to produce the SULT2B1b mRNA in human and mouse species is no longer required in the rat. The mechanism behind this precise exonic cutting and rearrangement is not presently understood.

Considering structure/function relationships involving SULT2B1 isoforms of the three species studied to date reveals consistencies as well as differences. For instance, the SULT2B1a isoform in the rat and human does not sulfonate cholesterol to any significant degree, whereas the SULT2B1a isoform in the mouse sulfonates cholesterol with an efficiency that is greater than that for either pregnenolone or DHEA (Shimizu et al., 2003). On the other hand, the SULT2B1a isoform in the rat as well as human (Strott, 2002) and mouse (Shimizu et al., 2003) more vigorously sulfonates pregnenolone by far over that of DHEA and cholesterol. In contrast, the SULT2B1b isoform in all three species sulfonates cholesterol with the highest efficiency and thus represents the physiologic cholesterol sulfotransferase. An outstanding structural feature of the SULT2B1 steroid/sterol sulfotransferases is an increase in their overall lengths when compared to prototypical SULT2A1 steroid sulfotransferases, as well as other cytosoloic sulfotransferases, an increase that is primarily due to extended amino- and carboxy-terminal regions of the SULT2B1 proteins (Strott, 2002). Interestingly, the unique amino-terminal region of the SULT2B1b isoforms in the three species is similar in size and amino acid composition. Based on deletion studies of the human SULT2B1 isoforms, it is the unique amino-terminal end of the SULT2B1b isoform, encoded by exon IB, that is important for cholesterol catalysis, i.e. if this section is removed, the ability of SULT2B1b to sulfonate cholesterol is lost (Fuda et al., 2002). Additionally, it is important to note that the amino acid residues within the amino-terminal end of human SULT2B1b determined to be essential for cholesterol catalysis by mutational analysis, although not identical within the rat and mouse SULT2B1b proteins, are, nevertheless, composed of conservative substitutions (cf. Fig. 3). While the unique amino-terminal ends of the SULT2B1b protein of rat, mouse and human are similar in size the unique amino termini of rat and mouse SULT2B1a are markedly longer than that of human SULT2B1a. In contrast to the situation with the SULT2B1b isoform, the functional significance of the unique amino-terminal end of the SULT2B1a isoform is not appreciated. Unlike the SULT2B1b isoform, this section of human SULT2B1a could be removed without any affect on its ability to sulfonate pregnenolone (Fuda et al., 2002).

There are also consistencies and differences in the quantitative tissue expression of mRNAs for the SULT2B1 isoforms in rat (this publication), mouse (Shimizu et al., 2003) and human (authors' unpublished data) species. For example, in all three species, skin is the dominant tissue to express the SULT2B1b isoform. This is consistent with the role of cholesterol sulfate in epidermal differentiation and development (Strott and Higashi, 2003). Other human tissues to prominently express SULT2B1b are the placenta and prostate, whereas in the rat, placental tissue barely expresses SULT2B1b and the prostate appears not to express it at all; there is, however, modest expression of SULT2B1b in the mouse prostate. After skin, the next tissue of importance in SULT2B1b expression in the rat is the intestine; this is also the case in the mouse, whereas the human intestine appears to weakly express SULT2B1b. The third tissue in the rat to at least modestly express SULT2B1b is the kidney; this is also true for the human, whereas in the mouse kidney SULT2B1b is poorly expressed. Regarding expression of SULT2B1b in the rat kidney and prostate, male and female kidneys were found to contain high concentrations of cholesterol sulfate, whereas the concentration of cholesterol sulfate in the rat prostate was low (Iwamori et al., 1976), findings essentially consistent with the level of SULT2B1b mRNA expression in these tissues. The SULT2B1a isoform in the rat is expressed only in the brain and testis. In the mouse, SULT2B1a is almost exclusively expressed in the brain; on the other hand, the adult human brain appears not to express either of the SULT2B1 isoforms [standard RT–PCR suggested that human fetal brain may express SULT2B1a (Geese and Raftogianis, 2001)]. The significance of expression of SULT2B1a in brain tissue is the accepted importance of pregnenolone sulfate functioning as a neurosteroid, at least in the rodent brain (Baulieu, 1998; Alomary et al., 2001; Engel and Grant, 2001; Plassart-Schiess and Baulieu, 2001). It is the expression of SULT2B1a in the rat brain that has a particular interest for us, and we are now proceeding to further examine its expression in specific types of brain cells.

References

Alomary, A.A., Fitzgerald, R.L., Purdy, R.H., 2001. Neurosteroid analysis. Int. Rev. Neurobiol. 46, 97–115.

Baulieu, E.E., 1998. Neurosteroids: a novel function of the brain. Psychoneuroendocrinology 23, 963–987.

Baulieu, E.E., Robel, P., Schumacher, M., 2001. Neurosteroids: beginning of the story. Int. Rev. Neurobiol. 46, 1–31.

Engel, S.R., Grant, K.A., 2001. Neurosteroids and behavior. Int. Rev. Neurobiol. 46, 321–348.

Fuda, H., Lee, Y.C., Shimizu, C., Javitt, N.B., Strott, C.A., 2002. Mutational analysis of human hydroxysteroid sulfotransferase SULT2B1 isoforms reveals that exon 1B of the *SULT2B1* gene produces cholesterol sulfotransferase, whereas exon 1A yields pregnenolone sulfotransferase. J. Biol. Chem. 277, 36161–36166.

Geese, W.J., Raftogianis, R.B., 2001. Biochemical characterization and tissue distribution of human SULT2B1. Biochem. Biophys. Res. Commun. 288, 280–289.

Her, C., et al., 1998. Human hydroxysteroid sulfotransferase SULT2B1: two enzymes encoded by a single chromosome 19 gene. Genomics 53, 284–295.

Higashi, Y., et al., 2004. Expression of cholesterol sulfotransferase (SULT2B1b) in human skin and primary cultures of human epidermal keratinocytes. J. Invest. Dermatol. 122, 1207–1213.

Iwamori, M., Moser, H.W., Kishimoto, Y., 1976. Cholesterol sulfate in rat tissues. Biochim. Biophys. Acta 441, 268–279.

Javitt, N.B., Lee, Y.C., Shimizu, C., Fuda, H., Strott, C.A., 2001. Cholesterol and hydroxycholesterol sulfotransferases: identification, distinction from dehydroepiandrosterone sulfotransferase, and differential tissue expression. Endocrinology 142, 2978–2984.

Kakuta, Y., Pedersen, L.G., Carter, C.W., Negishi, M., Pedersen, L.C., 1997. Crystal structure of estrogen sulphotransferase. Nat. Struct. Biol. 4, 904–908.

Mount, S.M., 1982. A catalogue of splice junction sequences. Nucleic Acids Res. 10, 459–472.

Nagata, K., Yamazoe, Y., 2000. Pharmacogenetics of sulfotransferase. Annu. Rev. Pharmacol. Toxicol. 40, 159–176.

Pedersen, L.C., Petrotchenko, E.V., Negishi, M., 2000. Crystal structure of SULT2A3, human hydroxysteroid sullfotransferase. FEBS Lett. 475, 61–64.

Pedersen, L.C., Petrotchenko, E., Shevtsov, S., Negishi, M., 2002. Crystal structure of the human estrogen sulfotransferase–PAPS complex. J. Biol. Chem. 277, 17928–17932.

Plassart-Schiess, E., Baulieu, E.E., 2001. Neurosteroids: recent findings. Brain Res. Rev. 37, 133–140.

Shimizu, C., Fuda, H., Yanai, H., Strott, C.A., 2003. Conservation of the hydroxysteroid sulfotransferase *SULT2B1* gene structure in the mouse: pre- and postnatal expression, kinetic analysis of isoforms, and comparison with prototypical SULT2A1. Endocrinology 144, 1186–1193.

Strott, C.A., 2002. Sulfonation and molecular action. Endocr. Rev. 23, 703–732.

Strott, C.A., Higashi, Y., 2003. Cholesterol sulfate in human physiology: what's it all about? J. Lipid Res. 44, 1–11.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.